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NOTE ON

GYNOCARDIN AND GYNOCARDASE

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CXVI.—*Note on Gynocardin and Gynocardase.*

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The cyanogenetic glucoside gynocardin was first isolated by Power and Gornall (Proc., 1904, **20**, 137) from the seeds of *Gynocardia odorata*, R. Br., and has more recently been obtained by A. W. K. de Jong from the leaves of *Pangium edule*, Reinw. (*Rec. trav. chim.*, 1909, **28**, 24).

Power and Lees (Trans., 1905, **87**, 349) have shown that gynocardin possesses the formula $C_{13}H_{19}O_9N$, and that it undergoes hydrolysis according to the following equation:

$$C_{13}H_{19}O_9N + H_2O = C_6H_8O_4 + C_6H_{12}O_6 + HCN.$$

The last-mentioned investigators, however, found that, of the three hydrolytic products, only dextrose and hydrogen cyanide could be characterised, as the other product spontaneously resinified.

The present authors thought that some further information regarding the unknown hydrolytic product of gynocardin might be obtained by methylating gynocardinic acid (Power and Lees, *loc. cit.*) according to Purdie and Pitkeathly's method (Trans., 1899, **75**, 153), and subsequently hydrolysing the methylated product. Although the object in view has not been achieved, some new observations regarding gynocardin have been made which are considered worthy of record.

By the methylation of gynocardinic acid, a liquid *methyl* derivative was obtained which could be distilled without undergoing decomposition, and, on analysis, gave figures corresponding with the formula $C_{12}H_{14}O_4(OMe)_5{\cdot}CO_2Me$. The yield of this derivative was, however, small, and no definite hydrolytic product could be obtained from it.

Gynocardin has been found to possess feebly acidic properties. It is slightly acid to litmus, and yields with sodium ethoxide or sodium hydroxide a *sodium* derivative, $C_{13}H_{18}O_9NNa$.

It has been shown by Power and Lees (*loc. cit.*) that acids hydrolyse gynocardin only with difficulty, and the present authors thought that this might be owing to the fact that gynocardin was an α-glucoside, since E. F. Armstrong (*Proc. Roy. Soc.*, 1904, **74**, 188) has shown that such compounds undergo hydrolysis by means of dilute mineral acids more slowly than do their β-isomerides.

Quantitative experiments have now shown that, whereas gynocardin is rapidly hydrolysed by the enzyme gynocardase, which accompanies it in the seeds, it is comparatively indifferent towards emulsin. The converse of this is the case with amygdalin, this glucoside being only very slowly hydrolysed by gynocardase.

The behaviour of gynocardase towards salicin and maltose was then investigated, when it was ascertained that this enzyme caused only an extremely slow hydrolysis of the glucoside, but was absolutely without action on maltose. The effect of diastase on gynocardin was then examined, and it was observed that this enzyme was quite devoid of action on the glucoside in question, thus differing from emulsin, which does hydrolyse gynocardin, although only to a very minor extent.

When, however, the respective effects of gynocardase and emulsin on *l*-mandelonitrile glucoside were investigated, it was found that, in this case, the two enzymes possessed practically equal activity.

It is evident, therefore, that the glucoside, gynocardin, and the enzyme, gynocardase, must both belong to the β-series, notwithstanding the fact that emulsin is almost indifferent towards gynocardin, and that gynocardase has only a very slight action on amygdalin.

Quantitative experiments have also shown that the rate of hydrolysis of gynocardin by acids, although very considerably less than that of salicin, is actually greater than that of amygdalin.

It would appear, therefore, that gynocardin and gynocardase are analogous in their behaviour to phaseolunatin and its corresponding enzyme, which were originally assigned by Dunstan and Henry (*Proc. Roy. Soc.*, 1903, **72**, 285) to the α-series, but have quite recently been shown by H. E. Armstrong and E. Horton (*ibid.*, 1910, **28**, *B*, 349) to belong to the β-series.

The fact that the enzyme from *Phaseolus lunatus* seeds is quite active towards *l*-mandelonitrile glucoside, whilst it exerts only a very minor action on amygdalin, was attributed by Armstrong and Horton (*loc. cit.*) to the inhibiting action of the second glucose molecule in the last-mentioned compound. Gynocardase, however, although behaving quite analogously to phaseolunatase with respect to amygdalin and *l*-mandelonitrile glucoside, only causes an extremely slow hydrolysis of salicin. It is evident, therefore, that the nature of the non-glucose part of the molecule of a glucoside may also exert a considerable influence with regard to the susceptibility of such a compound to the action of enzymes.

Experimental

Methylation of Gynocardinic Acid.

Gynocardinic acid was prepared by the action of hot barium hydroxide on gynocardin, when ammonia is evolved and barium gynocardinate formed (Power and Lees, *loc. cit.*). The barium gynocardinate thus obtained is a readily soluble salt, from which the gynocardinic acid may be prepared by exactly precipitating

the barium with sulphuric acid. It was observed by the present authors that when a considerable excess of barium hydroxide is employed in the hydrolysis a very sparingly soluble crystalline double compound of barium gynocardinate and barium hydroxide separates, but this was not further investigated.

Twenty grams of gynocardinic acid were methylated by means of methyl iodide and dry silver oxide according to Purdie and Pitkeathly's method (*loc. cit.*). The methylation was first carried out in methyl alcohol solution, and was repeated under these conditions until the product was soluble in methyl iodide, after which further methylation was effected in methyl iodide solution, the treatment being continued until there was no further reaction on boiling with dry silver oxide. The methylated product was removed from the reaction mixture by means of ether. It formed a syrup, which showed no signs of crystallising. When distilled under diminished pressure, a definite fraction boiling at 220°/15 mm. was obtained, which formed a viscid liquid at the ordinary temperature:

0·1642 gave 0·3170 CO_2 and 0·1145 H_2O. C = 52·6; H = 7·7.
$C_{19}H_{32}O_{11}$ requires C = 52·3; H = 7·3 per cent.

A determination of the number of methoxyl groups by Perkin's modification of Zeisel's method gave the following result:

0·1880 gave 0·6010 AgI. OMe = 42·2.
$C_{13}H_{14}O_5(OMe)_6$ requires OMe = 42·6 per cent.

This substance is therefore a *methyl pentamethylgynocardinate*, $C_{12}H_{14}O_4(OMe)_5 \cdot CO_2Me$, and must therefore still contain two hydroxyl groups, since Power and Lees have shown that gynocardin yields a hepta-acetyl derivative. In the endeavour to obtain a fully methylated product, a small quantity of the ester was heated with methyl iodide and dry silver oxide for some hours at 100° in a sealed tube, but the product gave practically the same results on analysis as before this treatment.

On boiling methyl pentamethylgynocardinate with dilute sulphuric acid, it was completely decomposed, nothing but brown, resinous products resulting.

Sodium Derivative of Gynocardin, $C_{13}H_{18}O_9NNa$.

As already mentioned, gynocardin possesses feebly acidic properties, in virtue of which it is able to form derivatives with the alkali metals. On adding excess of sodium ethoxide or aqueous sodium hydroxide to an ethyl-alcoholic solution of gynocardin, a white precipitate is produced, readily soluble in water or methyl alcohol, but sparingly so in ethyl alcohol:

0·4140 gave 0·0635 Na_2CO_3. Na = 6·6.

$C_{13}H_{18}O_9NNa$ requires Na = 6·5 per cent.

Attempts to prepare methyl gynocardin from this *sodium* derivative were unsuccessful.

Action of Gynocardase and Emulsin on Gynocardin and other Glucosides.

As stated above, gynocardin is but slowly hydrolysed by boiling dilute mineral acids, although this change is rapidly effected by means of the enzyme, gynocardase, which was obtained from *Gynocardia* seeds by Power and Lees (*loc. cit.*). Amygdalin, on the other hand, is only very slowly acted upon by gynocardase, the action requiring about twelve hours before any benzaldehyde and hydrogen cyanide can be detected. The effect of gynocardase and emulsin on gynocardin and amygdalin has therefore been examined quantitatively. In each case 0·5 gram of the glucoside was dissolved in 50 c.c. of water and 0·2 gram of enzyme added, the mixture being allowed to remain for thirty minutes at 25°. The resulting hydrogen cyanide was then removed by a current of steam, and estimated by titration with silver nitrate in presence of a little sodium chloride, in the usual manner.

The results of these experiments are shown in the following table:

Enzyme.	Glucoside.	Volume of *N*/10-$AgNO_3$ required.	Percentage of glucoside hydrolysed.
Gynocardase	Gynocardin	4·0 c.c.	57·6
Gynocardase	Amygdalin	< 0·1 ,,	none measurable
Emulsin	Gynocardin	< 0·1 ,,	,, ,,
Emulsin	Amygdalin	4·8 ,,	88·1 ,,

The effect of the two enzymes in question on salicin was also investigated, and for this purpose one gram of the glucoside was dissolved in 100 c.c. of water, and 0·5 gram of the respective enzyme added. The mixture was then allowed to remain at 20° for thirty minutes, after which the saligenin formed was isolated by means of ether, and weighed. The results obtained were as follows:

One gram of salicin, on treatment with gynocardase, gave 0·0050 of saligenin. Percentage of glucoside hydrolysed = 1·2.

One gram of salicin, on treatment with emulsin, gave 0·2100 of saligenin. Percentage of glucoside hydrolysed = 48·4.

The relative activity of emulsin and gynocardase towards *l*-mandelonitrile glucoside was ascertained in the following manner. A sufficient supply of the pure glucoside not being available, an extract of the bark of *Prunus serotina*, Ehrhart, was employed, as this has been shown to contain *l*-mandelonitrile glucoside (Power and Moore, Trans., 1909, **95**, 243). One hundred grams of the

finely powdered bark were brought into 5 litres of boiling water, the mixture boiled for some time, and then filtered. The filtrate was evaporated under diminished pressure to 200 c.c., and then divided into two equal portions, to one of which 0·4 gram of gynocardase was added, and to the other the same amount of emulsin. Each of the mixtures was then kept for thirty minutes, after which the liberated hydrogen cyanide was estimated as before described. It was then found that the portion treated with gynocardase required, on titration, 1·6 c.c. of *N*/10-silver nitrate solution to produce a permanent turbidity, whilst the other mixture, which contained emulsin, required an almost identical amount, namely, 1·55 c.c.

Acid Hydrolysis of Gynocardin, Amygdalin, and Salicin.

The respective rates of hydrolysis by acids of gynocardin, amygdalin, and salicin were estimated quantitatively, as follows.

Equimolecular quantities of the three glucosides (1·00, 1·32, and 0·80 grams respectively) were boiled with 200 c.c. of a 2 per cent. aqueous solution of sulphuric acid. In the case of the two cyanogenetic compounds, steam was passed through the mixture for two hours, the liquid being maintained at practically a constant volume, after which the hydrogen cyanide in the distillate was estimated in the usual manner. In the case of salicin the mixture was heated for one hour in a flask attached to a reflux condenser, and the saligenin then isolated by means of ether, and weighed. It was thus found that, under analogous conditions, the extent to which these three glucosides were hydrolysed was as follows: Gynocardin, 6·5 per cent.; amygdalin, 5·0 per cent.; salicin, 78·7 per cent.

A quantity of gynocardin was dissolved in water, and diastase added, but, even after ten days, no hydrogen cyanide was developed. Similarly, on treating maltose with gynocardase for twenty-four hours, no change occurred, for, on subsequently converting the sugar into osazone, the product was found to consist solely of maltosazone.

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